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Induced Resistance: a Tool for Fungicide Resistance Management†

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Abstract: Induced resistance to the apple scab fungus *Venturia inaequalis* was demonstrated in greenhouse tests with 12-day-old seedlings of the apple cultivar Golden Delicious treated with methyl 2,6-dichloro-isonicotinate or 3,5-dichloro-salicylic acid prior to inoculation with the causal fungus. Studies of the dose-response of flusilazole on induced resistant plants revealed synergistic effects between both crop protection principles. Therefore, the use of such resistance-inducing compounds in the field might allow a reduction in the number of fungicide applications, and possibly a reduction in dose, thus resulting in improved efficacy of fungicides. There was also evidence that induced resistance could prove to be a valid strategy for the treatment of pathogen populations with reduced sensitivity to a given fungicide. © 1998 SCI

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Key words: induced resistance; integrated pest management; fungicide resistance management; *Venturia inaequalis*; apple scab

1 INTRODUCTION

Despite the wide implementation of modern concepts of integrated crop protection, there are still crops where intensive fungicide applications are needed to secure a high quality harvest.[1–3] Besides constituting an unavoidable burden for the environment and creating problems with residues in the food and consumer concern, frequent fungicide treatments greatly increase the risk of the development of pathogen populations resistant to the active ingredients. The utilisation of host resistance is a valuable tool for the implementation of both integrated crop-management and anti-resistance strategies, as it may reduce the frequency of fungicide applications.[4–6] However, the use of inbred host resistance has limitations. This type of resistance is parasite-specific, often ephemeral, and it is time-consuming to introduce the resistance genes into the various well-established commercial varieties.[7]

An increased level of resistance to a broad spectrum of pathogens can be achieved quickly through the application of chemicals acting as resistance inducers. This ‘induced resistance’ in the host plants results in a significant delay of disease epidemics and, therefore, in a reduction of crop losses.[8]

Using *Venturia inaequalis* (Cke.) Wint., the cause of apple scab and a pathogen which necessitates many fungicide applications to apple trees, we explored the concept of induced resistance as a new tool for fungicide resistance management.

2 Experimental

2.1 Test method

Experiments were carried out with highly susceptible 12-day-old apple seedlings (*Malus x Domestica* Borkh.

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cv. 'Golden Delicious') grown as indicated elsewhere.[9] Resistance was induced, either with methyl 2,6-dichloroisonicotinate (DCINA), or 3,5-dichlorosalicylic acid (DCSA). Droplets of solutions of these chemicals (20 μl) were applied between the cotyledons and the stem, and seven days later, plants were inoculated with conidial suspensions of *V. inaequalis* (10^5 ml^{-1}) and incubated as indicated elsewhere.[9] SBI sensitivity *in vivo* was determined in greenhouse experiments by spraying different concentrations of flusilazole ('Benocap' 20WG, DuPont de Nemours) onto apple seedlings one day before inoculation with the fungus. The area of leaf showing infection was assessed nine days later. Four *V. inaequalis* strains, designated A, B, C and D, with different sensitivities to flusilazole were used for the experimental work. Their EC_{50} values determined *in vivo* on apple seedlings were 1·0, 1·6, 7·0 and 35·7 mg litre^{-1}, respectively.[10] The Wilcoxon rank-sum test was used for the statistical evaluation of the efficacy of induced resistance against these different strains.

2.2 Evaluation of fungicide dose-response curves

For the evaluation of the fungicide sensitivity, efficacy values were fitted to sigmoid curves with the formula

$$Y = (1 - e^{-(\log(X)/K)^{\beta}}) \cdot 100$$

where Y is efficacy and X is dose, by non-linear regression according to Ortega et al.[9,10] EC_{50} values, together with their confidence intervals at $\alpha = 0{\cdot}5$, were computed by inverse prediction in the non-linear regression process. For the calculation of the efficacy of the fungicide on induced-resistant apple plants, the induced but not fungicide-treated variant was used as reference for the calculation of the efficacy as percentage control of infection.

3 RESULTS AND DISCUSSION

The results in Fig. 1 indicate that resistance to apple scab can be successfully induced in apple seedlings by application of DCINA or DCSA. This adds apple to the large number of crops where there is potential for induced resistance in practical crop protection.

To evaluate the use of induced resistance as tool in fungicide resistance management strategies, it is important to study if there is any evidence of cross-resistance between the two control measures. Therefore, the efficacy of induced resistance against strains with varying sensitivity to DMI fungicides was investigated. Differences of up to 50% in the efficacy of induced resistance against distinct strains of *V. inaequalis* could be detected (Fig. 1). However, the level of sensitivity of these strains to triazoles was not correlated with the efficacy reached by induced resistance against them.

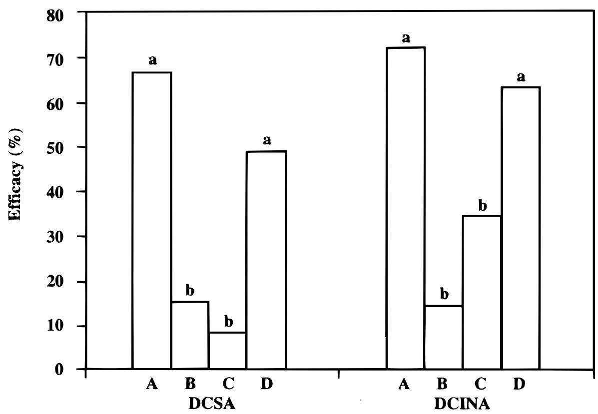

Fig. 1. Resistance against strains of *Venturia inaequalis* with differing sensitivities to triazole fungicides imparted to apple seedlings (cv. Golden Delicious) as a result of stem application of methyl 2,6-dichloroisonicotinate (DCINA; 500 mg litre^{-1}) or 3,5 dichlorosalicylic acid (DCSA; 350 mg litre^{-1}). Strain A (sensitive); Strain B (sensitive); Strain C (resistant) and Strain D (highly resistant). Columns of a given treatment which bear the same letter are not significantly different in the Wilcoxon rank-sum test, $\alpha = 0{\cdot}05$.

Therefore, the efficacy achieved by induced resistance against *V. inaequalis* is very much dependent on the pathogen genotype, and not on the level of sensitivity to triazoles. Most significantly, the level of protection against the strains on plants induced with either DCINA or DCSA was quite similar, probably because both inducing agents affect the same metabolic process in the plants, as postulated in literature.[11]

Under practical conditions plants are constantly challenged with genetically non-homogeneous pathogen populations. Therefore, the efficacy of induced resistance was tested against mixed populations of these *V. inaequalis* strains to study possible interactions between different strains in relation to the level of control reached by induced resistance. The efficacy of induced resistance against a mixed pathogen population of different genotypes was either comparable, or clearly superior, to that reached against the best-controlled

TABLE 1
Efficacy of DCINA-Induced Resistance against Individual Strains of *Venturia inaequalis* and Their Mixtures

Strain of V. inaequalis		*Efficacy of induced resistance* (%)[a]		
Strain 1	*Strain 2*	*Mixture*[b]	*Strain 1*	*Strain 2*
A	C	72a	48b	19c
A	D	63a	48ab	37b
B	C	48a	29a	28a
B	D	67a	29b	53a
C	D	29ab	19a	37b

[a] Values with the same letter on the same horizontal line are not significantly different at $\alpha = 0{\cdot}05$, Wilcoxon rank-sum test.
[b] Strains mixed in 1 : 1 ratio.

strain in the mixture (Table 1). This was true for all the combinations between the four strains studied. It seems that this effect could counter any possible selective pressure exerted by induced resistance on the pathogen populations but competitive studies *in vivo* between the different strains on induced-resistant plants are necessary to shed light on this important issue.

Due to its quantitative nature, induced resistance gives only a partial protection against diseases during the vegetation period. This implies that, in most crops, it will be necessary to use these agents in combination with fungicides to obtain a satisfactory level of protection. Therefore, it is important to investigate whether there are interactions between the activities of fungicides and induced resistance.

A clear effect of induced resistance on the response of the *V. inaequalis* strains to different dosages of the fungicide flusilazole was detected. The efficacy of the fungicide was enhanced on induced-resistant plants, as indicated by a modification of the dose-response relationship towards a higher activity of the fungicide (Fig. 2). The EC_{50} value of the fungicide against the highly triazole-resistant strain D of *V. inaequalis* shifted significantly from 35·8 to 10·1 mg litre^{-1}, when a flusilazole dose-response was tested on induced-resistant apple seedlings. Further, a maximum synergy ratio of $R = 1{\cdot}4$ could be calculated according to the method of Abbott.[12] This indicates that the interaction between induced resistance and flusilazole resembles synergistic effects observed for several xenobiotics. As the applications of DCINA and the fungicide were separated in space and in time, this effect must be due to a direct interaction between induced resistance and the fungicide, rather than to formulation effects. A similar enhancement of the efficacy of flusilazole due to induced resistance could be detected for other strains of *V. inaequalis*, independently of their level of sensitivity to triazoles.

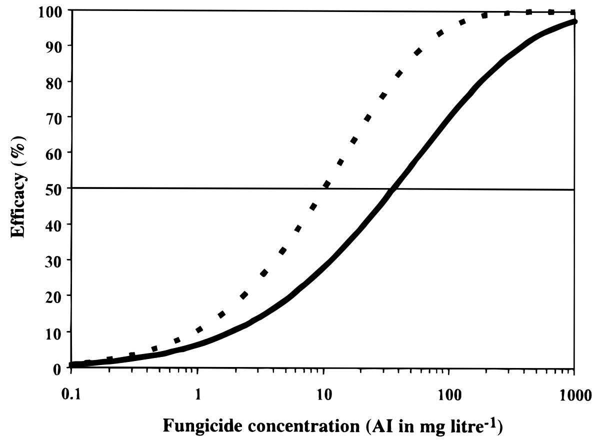

Fig. 2. Dose-response of the *Venturia inaequalis* strain D (highly resistant to triazoles) to the fungicide flusilazole on induced-resistant and non-induced apple seedlings. DCINA (250 mg litre^{-1}) was applied to stems of treated seedlings and treated and untreated seedlings were then inoculated with the fungus and subsequently sprayed and flusilazole (see text) using a range of concentrations. —— fungicides only (EC_{50} 35·8 mg litre^{-1}); - - - fungicide + DCINA (EC_{50} 10·1 mg litre^{-1}). The EC_{50} values are significantly different at $\alpha = 0{\cdot}05$ (inverse prediction by non-linear regression).

4 CONCLUSIONS

With its integration into practical crop-protection strategies, induced resistance could bring about a reduction of the number of fungicide applications and, perhaps, dose rate, and lead to an improvement of the efficacy of fungicides. Further, induced resistance was active against pathogen populations with a reduced sensitivity to fungicides. This suggests that induced resistance may be used as an anti-resistance strategy for fungicides in integrated crop protection.

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